Middle East Journal of Applied Science & Technology (MEJAST)
Volume 7, Issue 4, Pages 66-80, October-December 2024

Larvicidal, ovicidal and repellency properties of the indigenous fruit *Citrus reticulata* (Orange) peels essential oil against *Aedes aegypti* mosquitoes

Maung Maung Mya[1*], Saw Htet Thura Lin[2], Aye Win Oo[3], Than Myat Htay[3], Chit Thet Nwe[1], Thu Zar Nyein Mu[1], Zar Zar Aung[1], Yee Yee Myint[1] & Sein Thaung[1]

[1]Medical Entomology Research Division, Department of Medical Research, Myanmar. [2]Yangon University, Myanmar. [3]Animal House, Department of Medical Research, Myanmar. Corresponding Author (Maung Maung Mya) Email: dr.mgmgmya@gmail.com[*]

DOI: https://doi.org/10.46431/MEJAST.2024.7406

Article Received: 15 September 2024 *Article Accepted: 21 November 2024* *Article Published: 28 November 2024*

ABSTRACT

Mosquito-borne diseases are mostly harmful to children being and it is a public health problem in Myanmar. Laboratory reared Insein strain of *Aedes aegypti* larvae were used to test larvicidal, ovicidal and repellency properties of *Citrus reticulata* (Orange) peels essential oil from June 2020 to May 2021 according to WHO. Fresh peels 300 grams from Aung Ban was extracted by stream distillation at 100 °C for 3hours and obtained 1.70g of essential oil. Different concentrations of orange peels essential oil were prepared freshly in 100ml each of distilled water in 150ml plastic cups. Fifty each *Aedes* larvae were exposed 24hrs for each replication in different concentrations in laboratory. Acute toxicity and allergenicity tests were done in laboratory according to OECD Guidelines. Repellency test was done by laboratory reared 5-7 days old adult female *Aedes* mosquitoes with orange peels essential oil. Results revealed that the highest dose 0.01g of orange peels essential oil produced 100% knockdown within 60minute and 100% mortality within 24hrs respectively and 100% ovicidal effect for 4days as well as persistency was observed 100% mortality of larvae for 4 days. The effective lethal concentrations LC_{50} and LC_{90} values were found to be 0.0015g and 0.0051g of peels essential oil (x=0.0418, P=0.05). There was not found any acute toxicity on mice and allergenicity on the rabbits. 100% protection of *Aedes* mosquito landing to probe the skin was found 0.04g/ml or 0.000128g/cm^2 of essential oil. Repellency activity of complete protection time was observed over 80% protection for 210minutes, over 90% prevention for 150 minutes, and 100% prevention for 120 minutes. Semi field trial observed that in the day time, it can prevent 3 hours of *Aedes* mosquito bite on oil applied areas of insect collectors in household. The essential oil is not toxicity, no allergenicity and no irritation of skins of animal. Hence, the cheap, effective, ecofriendly and degradable Orange *Citrus reticulata* peels essential oil can be used as insecticide and repellent of mosquitoes in public sector.

Keywords: Orange peel; Larvicidal; Ovicidal; Repellency; Allergenicity; Toxicity; LC_{50}; LC_{90}; Mortality; Knockdown; Essential oil.

1. Introduction

Three main groups of mosquitoes which are *Aedes*, *Culex* and *Anopheles*. *Aedes aegypti* is one of the faster allocated mosquitoes and mostly found in tropical and sub-tropical Regions in the world and responsible vector for Dengue Fever (*DF*), Dengue Hemorrhagic Fever *(DHF*), Zika, Chikungunya and Yellow fever (1Service 1993). Dengue is an arbovirus disease it coursed *DF* and *DHF* and its subsequent Dengue Shock Syndrome (*DSS*) and have four serotypes of dengue virus. *Culex* species are also important targets for vector control and for the prevention of arboviruses, including Japanese encephalitis virus (JEV) and West Nile virus avian (WNV) (Service 19930. *Culex quinquefasciatus* transmit filariasis in men Avian malaria in birds and *Culex tritaeniorhynchus* transmitted Japanese encephalitis. *Anopheles dirus* and *An. minimus* transmit malaria (Kyi 1970, Mya et al., 2021).

World Health Organization revealed that 7.6 million dengue cases were reported in 2023, including 3.4 million confirmed cases, over 16000 severe cases, and over 3000 deaths. While a substantial increase in dengue cases has been reported globally in the last five years (WHO 2023). In India, dengue fever and Chikungunya are main Public Health Problem (Nathan 2006). DHF, malaria, filariasis are harmful to human and they are a public health problem in Myanmar. Union Health Ministry reported that considerably increase the number of cases in Yangon, Mandalay, Mawlamyaing and Hpa-an in last few years (MOH 2020). A severe outbreak of DHF occurred for the first time in Yangon in 1970. The highest number of cases and deaths recorded were 9149 DHF cases and 55 deaths recorded across Myanmar (DOPH 2016). Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) are increasingly

becoming serious public health problem in Myanmar, and different types of controlling techniques are using in different parts of Myanmar. Vector Borne Diseases control programme used Abate in water storage containers in all parts of State and Regions (VBDC 2020).

Previously, vector control approaches could be divided into three general categories: chemical control, biological control, and environmental management, regardless of the pathogens to be targeted. All control strategies share a common goal of reducing the sizes of vector populations. The fundamental approach for chemical control is through the release of insecticidal chemical compounds, which inevitably faces the challenge of insecticide resistance and off-target effects on other arthropod species. Extensive use of chemical insecticides for control of vector borne diseases has created problems related to physiological resistance to vectors, adverse environmental effects, high operational cost and community acceptance (Kim et al., 2001).

And also, the use of indoor residual spray is more likely to be effective in controlling indoor species such as *Ae. aegypti* but has limited impact on the populations of various *Culex* species mosquitoes, which rest outdoors (Warikoo et al., 2011). Moreover, Chemical and synthetic organic insecticides have been used for mosquitoes' control and these are toxic to environment, human being, soil, water and air. Biological control depends on the use of predatory or parasitic organisms targeting disease vectors. Additionally, the removal of habitats in the environment can aid in the population reduction of disease vectors (Mya et al., 2011).

Although some were used biological control methods as introducing the larvivorus fishes *Gambusia*, *Applochilus panchus*, and dragon fly nymphs were found very effective larval control tools and found eco-friendly, cheeps and degradable (Mya et al., 2013,2016) and recently used most popular biological control technique as introducing the Wolbachia bacteria, which has enhanced replacement programs, and the introduction of dominant lethal genes into local mosquito populations through the release of genetically modified mosquitoes (Ogunlade et al., 2022). Transmission of the disease can be reduced by community participation in vector control. Dengue is an arbovirus disease it coursed *DF* and *DHF* and its subsequent Dengue Shock Syndrome (*DSS*) and have four serotypes of dengue virus. Transmission of the disease can be reduced by community participation in vector control.

As various mosquito species and arboviruses continue to expand to new areas, there will be a continuing need for new biological control strategies. Although the goal of completely eliminating disease vectors is impractical, it has always been true that the reduction in the numbers of competent vectors is an important part of disease control (Huang et al., 2020). *Citrus* species (Rutaceae), native of the tropical regions of South-east Asia and China, is a great source of EOs which possesses numerous oil. Orange *Citrus reticulata* L., is a plant native to tropical Asia, Africa and the Pacific Islands. Fruit peels contains a high percentage of volatile and essential oils and is used in the conventional medicine to alleviate pain, vomiting and even as an insect repellent (Gazali et al., 1988).

Control of mosquito is essential as many species of mosquitoes are vectors of malaria, filariasis, and many arboviral diseases; and they constitute an intolerable biting nuisance Anthony & Service 1983. Biotechnologists and entomologists agree that mosquito control efficiency should be with selectivity for a specific target organism. New control methodologies aim at reducing mosquito breeding sites and biting activity by using a combination of chemical–biological control methods soothing several advocated biocontrol methods to reduce the population of mosquito and to reduce the man–vector contact (Service 1993). Recently, there has been a major concern for the

promotion of botanicals as environment friendly pesticides, microbial sprays, and insect growth regulators amidst other control measures such as beneficial insects and all necessitate an integration of supervised control (Ascher et al., 1995, Nathan 2004, 2005b, 2005c, Nathan & Kalaivani 2005). The development of insects' growth regulators (IGR) has received considerable attention for selective control of insect for medical and veterinary importance and has produced mortality due to their high neurotoxic effects (Wandscheer et al., 2004, Brown 1986). Numerous plant products have been reported either as insecticides for killing larvae or adult mosquitoes or as repellents for mosquito biting and are one of the best alternatives for mosquito control (Brown 1986, Sukumar et al., 1991).

Several studies showed that plant extracts or essential oils can provide desired larvicidal properties against different species of mosquitoes without causing bad effects to humans (Gimlette & Thomson 1983, Yeung 1985, Kiram et al., 2006). Mosquito larvicidal agents from plants and microorganisms can be used as effectively without causing any ill effects to living being. Also plant insecticides can be easily procured due to its natural source supply; they are economical and are ecofriendly. Bio based insecticides are generally nontoxic to human and environment (Mya et al., 2015, Ibrahim et al., 1996).

Studies found that *Citrus* family plant has enormous potential for controlling the population of larvae and adults of different types of mosquitoes. *Citrus reticulata, Citrus hystrix* DC. (Kaffir lime) is a member of *Citrus* family and its leaves, fruits, barks and roots are being used in tropical Asia for culinary and traditional medicine purposes. *Citrus reticulata* plants are also available in many parts of the Myanmar and plants are abundantly present in mountain areas of Shan State, Kachin, Sagaing, Chin and Kayar State.

The essential oils in some article reported to possess other activities such as antibacterial, antifungal, mosquito larvicidal and repellent (Dan B, 2004). However, there is no reports of *Citrus reticulata* fruit peels essential oil on *Aedes* mosquitoes has been mentioned as an insecticidal agent in Myanmar. Therefore, attempt has been made to investigate the larvicidal ovicidal and repellent action of *Citrus reticulata* fruit peels essential oil against *Aedes aegypti* larvae and adults to support the future vectors control activity in environmental sound manner.

1.1. Objectives of the Study

(i) To access larvicidal and ovicidal effect of *Citrus reticulata* peel essential oil.

(ii) To investigate the repellency effect of *Citrus reticulata* peel essential oil.

(iii) To measure the repellency effect on wild *Aedes* mosquitoes in simi field condition.

2. Materials and Methods

2.1. Mosquito larvae collection

Insein Township strain of *Aedes aegypti* mosquito larvae and adult *Aedes* mosquitoes emerged from pupae were reared in laboratory of Medical Entomology Research Division, Department of Medical Research. Larvae were feed on DMR larva food. Adult were provided with 10% sucrose solution and 8weeks old mouse for blood meal. Mosquitoes were held at 26±2 °C, 65-75% relative humidity with a photo period of 12–hours light and 12-hours dark. Laboratory reared larvae and mosquitoes were used for testing insecticidal properties of stream distillated essential oil of fresh *Citrus reticulata* L fruit peels which were collected from Aung Ban Township Shan State.

2.2. Species identification of mosquitoes

Larvae and adult mosquitoes emerged from larval survey were identified by morphological methods according to the key of Rampa and Prachong 1994.

2.3. Collection and preparation of essential oil extraction from *Citrus reticulata* fruit peels

Citrus reticulata L fruit peels Myanmar name Leainmawthii (Orange) was collected from Aung Ban Township, Shan State. A total of 5Kelo grams of *Citrus reticulata* L fruit peels was cleaned and 300 grams of *Citrus reticulata* L fruit peels were extracted with 1000ml distilled water by stream distillation method at 100°C for 3 hours. Complete removal of the solvent from the extract was accomplished in glass rotary evaporator. The resulting 1.70gm of essential oil was obtained from 300gm of fresh *Citrus reticulata* fruit peels. The essential was stored at 4°C in refrigerator until use. The extraction was done in Department of Pharmacology, Institute of Medical Technology, Yangon.

2.4. Larvicidal testing procedure

Based on preliminary tests, further *Citrus reticulata* L fruit peels essential oil as 0.1g, 0.05g, 0.025g, 0.0125g and 0.00625 g were prepared freshly in 100ml each of distilled water in 150ml plastic cups. Fifty (50) each 3rd and 4th instars *Aedes aegypti* larvae were put into different concentrations and negative control test was done simultaneously. Detail testing was done according to standard method (WHO2005). Larvae were exposed 24 hours for each replication in different concentrations in laboratory at 27-29 °C and 70%-80% relative humidity. Five replicates were carried out and knockdown was checked and recorded after 60 minutes exposure period and mortality was checked and recorded after 24 hours of exposure periods. Knockdown and dead larvae were identified when the larvae failed to move after probing with a needle in the thorax region of the body. Lethal concentration LC_{50} and LC_{90} values for 95% confidential limits were calculated by following formula (Finney 1971).

X2=(O-E)2/Ex(100-E) (X2= Chi squire, O=Observed value, E=expected value, O-E=Observed minus expected)

2.5. Repellent activity testing

The repellent study was followed the method of WHO 2005. Five days old blood staved female *Aedes aegypti* mosquitoes 60 each were kept in two steel net cages (59x59x59cm) one for male and one for female volunteers. *Aedes aegypti* is a day time biter; therefore, the tests were done between 08:00 hour and 16:00 hour. Evaluation tests were carried out in a (12x15x15ft) room at 24-28°C and relative humidity of 70-85%. The volunteers had no contact with lotions, perfumes, or perfumed soaps on the day of assay. The arms of volunteers, one ml of ethyl alcohol 95% diluent used in the preparation of the test repellent in applied evenly using a pipette to average 372.375cm^2 (Four volunteers-two males +two female) of forearm skin between the wrist and elbow and allowed to dry for 1 minute. Before insertion of arm into the cage containing 60 *Aedes* female mosquitoes, the hands are protected by plastic gloves to protect mosquito bite. The first step, ethyl alcohol applied forearm was inserted into the cage and counted the number of mosquitoes that land on skin during 30-secind period. The control forearm was carefully withdrawn and this arm was then treated with one ml of 0.04g/ml or 0.000128g/cm^2 of essential oil solution and allowed to dry. The treated arm was placed in the cage for another 30 second period and on served for

mosquito landing. This procedure was repeated for each additional incremental of *Citrus reticulata* peel essential oil dose. The tests were carried out one after the other without delay and *Citrus reticulata* fruit peels essential oil dose at each test was calculated as the sum of the doses applied to arrive at the cumulative dose for each test. Test was proceeding when the mosquito landing rate on the exposed forearm was less than 10 females in 30 second. Two trained technicians were recorded the number of landings. At the conclusion of the dose response experiment, 1ml of ethyl alcohol was applied on the other forearm and allowed to dry. This forearm was inserted in the cage for 30 second to verify that the number of landings was more than 10 per 30 second as was observed at the beginning of the experiment. Protection (P) was expressed as a proportion of the number of mosquito landing on treated arm (T) in relation to the number of landings on the control arm © of the same individual. Where C is the average of landing mosquitoes on two untreated arms.

P+1-(T/C)=(C-T)/C

2.6. Estimation of complete protection time

The complete protection time of *Citrus reticulata* fruit peels essential oil was determined 99 to 100% protection dose 0.0125g/ml, 0.025g/ml, 0.05g/ml and 0.1g/ml were using on average 372.375cm^2 areas (another 2 male and 2 female volunteers) of forearm skin between the wrist and elbow individually. The protection test was followed by the procedure described as above. Before testing two mosquito cages (size 59x59x59cm) each containing 60 non blood fed 5 days old *Aedes aegypti* female mosquitoes were normally used. One cage was used for testing female volunteers and another cage was used for testing male volunteers. Before testing the arms of volunteers, one ml of ethyl alcohol 95% diluent used in the preparation of test of *Citrus reticulata* fruit peels essential oil repellent in applied evenly using pipette to average 372.375cm^2 of forearm skin between the wrist and elbow and allowed to dry 1 minute. Before insertion of arm into the cage containing 60 *Aedes* female mosquitoes. The hands are protected by plastic gloves to protect mosquito bite. The first step, ethyl alcohol applied forearm was inserted in to the cage and counted the number of mosquitoes that land on the skin during 3minute period. The control forearm was carefully withdrawn from the cage. Then 0.1g of *Citrus reticulata* fruit peels essential oil was prepared in one ml of ethyl alcohol solution was applied evenly on average 372.375cm^2 of another forearm skin between the wrist and elbow. The treated arm was placed in the cage for 3minute period and observed for mosquito landing.

After 30 minutes, the *Citrus reticulata* fruit peels essential oil repellent treated arm was inserted again into the cage and exposed for 3 minutes to determine landing activity. This procedure was repeated at 30minute intervals for 240 minutes and the procedure was used consistently throughout the experiment. Test was repeated 4 times for 2 males and 2 females' volunteers. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing and blood. Test was proceeding when the mosquito landing rate on the exposed forearm was less than 80% within 3 minutes. The end of the experiment, ethyl alcohol applied forearm was inserted into the cage and counted the number of mosquitoes that land on the skin during 3-minute period to verify that the number of landings was same or not observed at the beginning of the experiment. The control forearm was carefully withdrawn from the cage. The average of landing mosquitoes on two untreated arms was used to calculate the percentage protection of mosquito bite. Complete protection time was estimated after experiment.

2.7. Data analysis plan

Data entry and proceeding was made using Microsoft Excel software. The average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90} values and other statistics at 95% confidence limits and Chi-squire values were calculated using the dose –effect probit analysis [34]. Results with $p<0.05$ were considered to be statistically significant.

3. Results

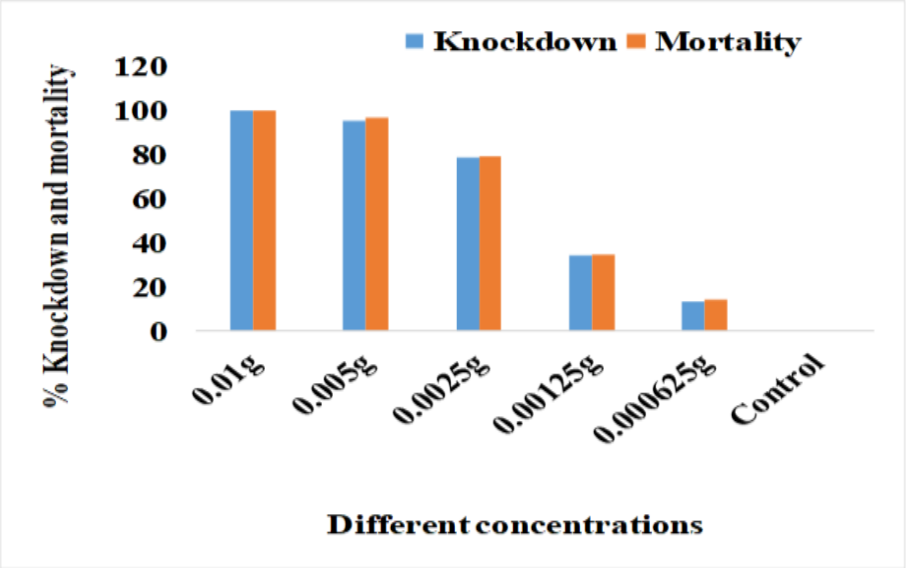

Figure 1. Knockdown and mortality effect of different concentrations of *Citrus reticulate* peels essential oil against 3rd and 4th instars *Aedes* larvae

Figure 1 shows that 100% knockdown and mortality of 3rd and 4th instar larvae found 0.01g dilution of Orange peels essential oil and followed by 95.6% knockdown and 97% mortality were found 0.005g dilution of Orange peels essential oil and lowest knockdown and mortality was observed on 0.000625g dilution of Orange peels essential oil.

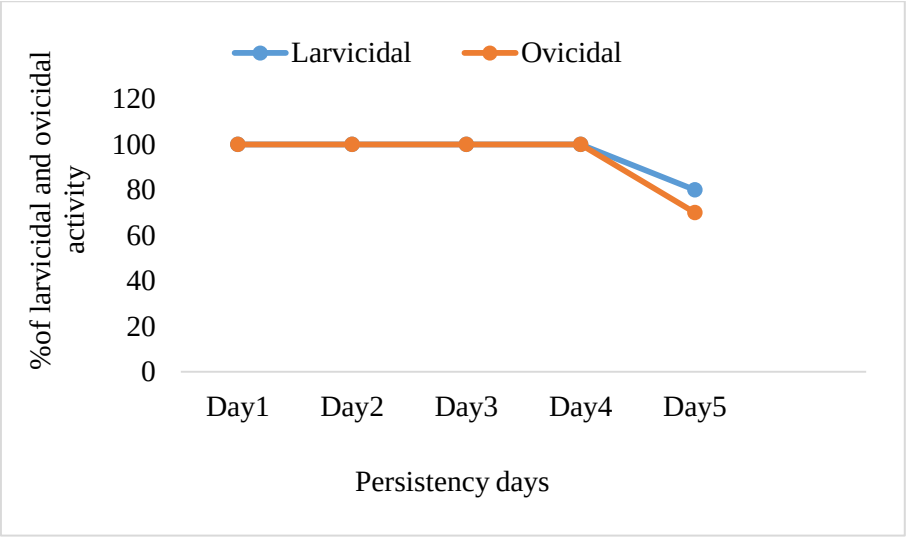

Figure 2. Larvicidal and ovicidal persistency of 0.01g *Citrus reticulata* peels essential oil against 3rd and 4th instar larvae and eggs

Figure 2 shows that Larvicidal and ovicidal persistency of 0.01g *Citrus reticulata* peels essential oil against 3rd and 4th instar larvae and eggs was found 4 days. After 4 days persistency of *Citrus reticulata* peels essential oil was fail down to 70 to 80% of larvicidal and ovicidal.

Table 1. LC_{50} and LC_{90} values of *Citrus reticulata* peels essential oil on 3rd and 4th *instar Aedes aegypti* larvae

Treatment	**Hours**	**Oil**	**X^2, P<0.05**	**Corrected Lethal Concentration LC_{50}**	**Corrected Lethal Concentration LC_{90}**
Citrus reticulata essential oil	24	Watery extract	X^2=0.418 P<0.05	0.0015g	0.0051g

Table 1 shows that LC_{50} and LC_{90} values of *Citrus reticulata* peels essential oil on 3rd and 4th instar *Aedes aegypti* larvae and found that 50% mortality of corrected lethal concentration LC_{50} was 0.0015g and 90% mortality of corrected lethal concentration LC_{90} was found 0.0051g.

Table 2. Acute toxicity effect of *Citrus reticulata* peels oil on albino mice model after two weeks of administration

No.	**Groups**	**Essential oil administration Dosage**	**Dosage**	**No. of death**	**% of death**
1	Group 1 10 mice	*Citrus reticulata* peel essential oil	2000 mg/kg	Nil	0%
2	Group 2 10 mice	*Citrus reticulata* peel essential oil	5000mg/kg	Nil	0%
3	Group 3 10 mice	No administration Nil (only clean water)	Nil	Nil	0%

Table 2 shows that acute toxicity effect of *Citrus reticulata* peels essential oil on albino mice model after two weeks of administration and found that there was not toxicity effect of *Citrus reticulata* peels essential oil dosages 2000mg/kg and 5000mg/kg against albino mice model after two weeks of administration.

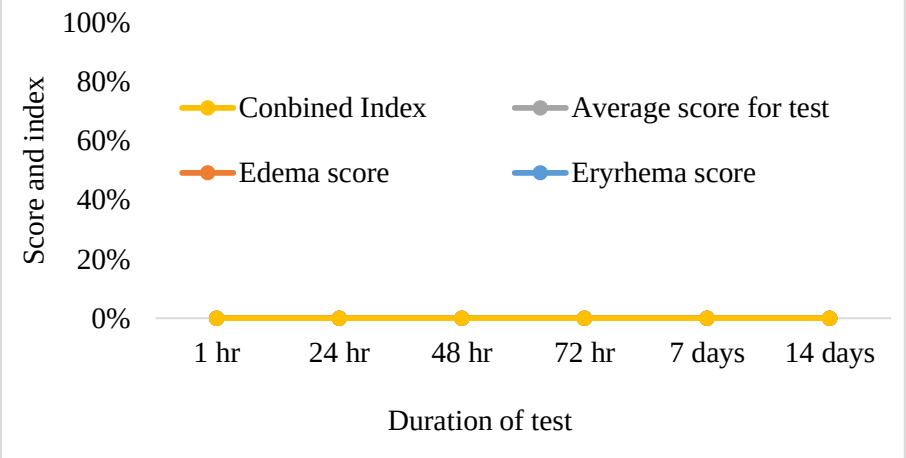

Figure 3. Allergenicity effect of orange peels essential oil reactions on rabbits

Figure 3 shows the allergenicity effect of orange peels essential oil reactions on rabbits and found that there was no allergenicity effect on Rabbet skin within 14 days.

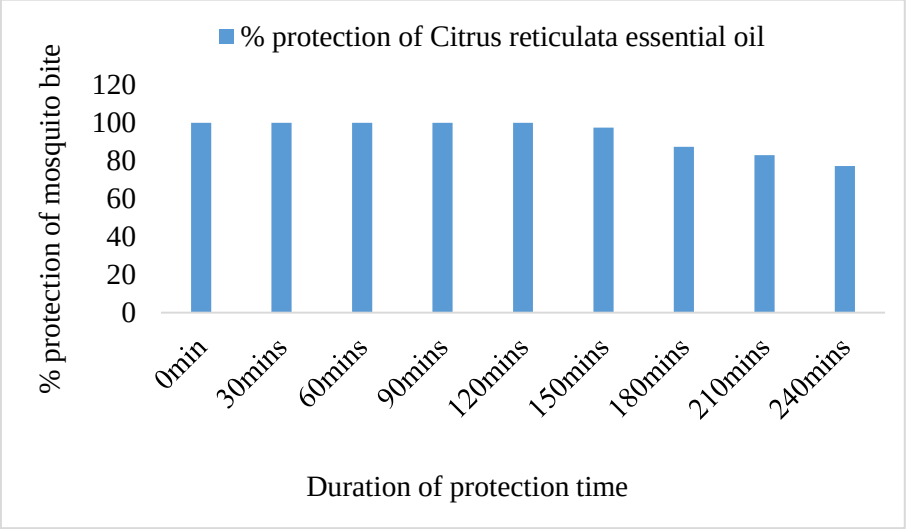

Figure 4. Complete protection time of orange peels essential oil against *Aedes* mosquito bite

Figure 4 shows complete protection time of orange peels essential oil against *Aedes* mosquito bite and found that 0.04g /ml or 0.000128g/cm^2 of *Citrus reticulata* essential oil was found very effective repel to protect 80 to 100% protection of *Ae. aegypti* mosquito bite for 210 minutes. 100% of protection of mosquito bite was found 120 minutes and over 90% protection was observed 150 minutes.

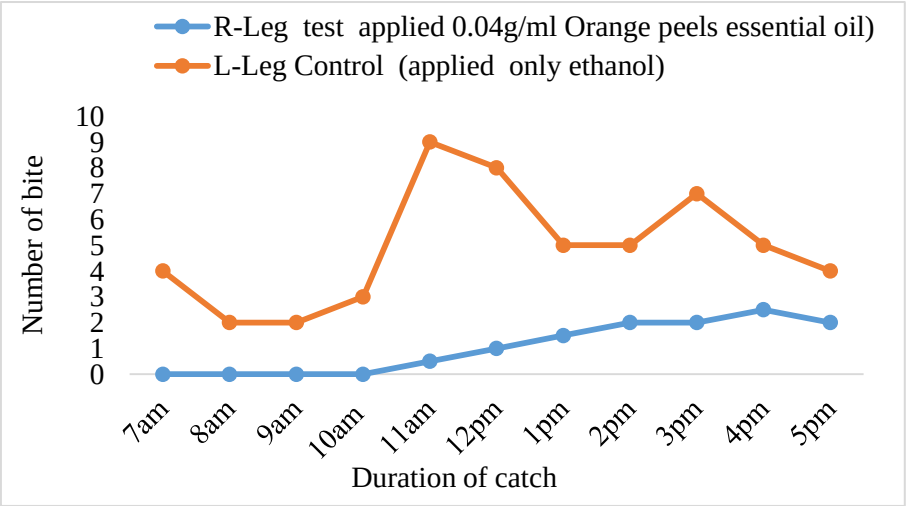

Figure 5. Hourly mean biting rate of *Aedes* mosquitoes on orange peels essential oil and only Ethanol applied areas of insect collators in simi field condition

Figure 5 shows hourly mean biting rate in day time of *Aedes* mosquitoes on orange peels essential oil and only Ethanol applied areas of insect collators in simi field condition and found that 100% prevention for 3 hours of *Aedes* mosquito bite on oil applied areas of insect collectors in bushes and household in the daytime.

4. Discussions

The main vector of dengue virus in Myanmar is *Aedes aegypti*. Rapid, poorly planned urbanization in association with weak regulatory policies for discharge of solid waste has resulted in the accumulation of solid waste which in

turn results in the accumulation of discarded containers in most developing countries. Poor water supply created a lot of water storage containers for storing water for multiple used. These accumulations have favored the establishment and geographic spread of *Aedes aegypti* mosquitoes. It discarded largely because of it anthropophilic feeding behavior, resting behavior inside houses and its capability to exploit most water holding containers for breeding. Dengue Hemorrhagic Fever (DHF) is one of the global health concerns in tropical area and an endemic disease in more than 110 countries in the worlds (Ranjit 2011). Approximately 2.5 billion people live in endemic countries of which about 1.8 billion (more than 70%) in Southeast Asia and the Western Pacific Region (Ferreira & Camara 2018. *Aedes aegypti* is a main vector of dengue virus in urban areas and *Aedes albopictus* is rural area vector.

In the present study 0.01 g dilution of orange peel essential oil found 100% knockdown and mortality and followed 0.005g dilution of essential oil was found over 95% knockdown and mortality. The persistency of 0.01g *Citrus reticulata* peels essential oil for larvicidal and ovicidal against 3rd and 4th instar larvae and eggs was found 4 days. Same knockdown and mortality of 3rd and 4th instar *Aedes* larvae results have been found by using 0.01g dilution of *Citrus hydric* fruits extract and *Ocimum basilicum* L leaves essential oil against *Aedes aegypti* mosquitoes (Mya et al., 2011, 2023). Natural products are safe for humans when compared to that of synthetic compounds. According to Bowers et al., (1995) the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. Different parts of the plants contain a complex of chemicals with unique biological activity (Govindarajan et al., 2008a,2008b) which is thought to be due to toxins and secondary metabolites which act as mosquitocidal agent (Niraimathi et al., 2010). *C. grandis* belongs to the family Rutaceae and members of this family are said to have potent larval, adulticidal and repellent activities against different species of mosquitoes (Shaalan et al., 2005).

The flavonoid compounds found in *Poncirus irifoliate* (faily) Rutaceae) exhibited potent activity against larvae and eggs of *Aedes aegypti* eggs where the lethal concentration LC_{50} and LC_{90} of the flavonoids ranged from 0.082 to 0.122 mg/L and 0.152 to 0.223 mg/L respectively (Rajkumar & Jebanesan 2004).

In the present study LC_{50} for 50% mortality and LC_{90} for 90% mortality values were found 0.0015g and 0.0051g. Same larvicidal effect has been found Orange, Lemon and Orange and lemon mixture of essential oil and laboratory base larvicidal experiment indicate that the higher essential oil concentration found to be the higher the larval mortality, the essential oil of Orange at concentration of 0.01g/100ml water caused 98.33% mortality rate of *Ae. aegypti* larvae and Lemon essential oil at concentration 0.01g/100ml water caused 100% mortality rate of *Ae. aegypti* larvae as well as Orange and Lemon essential oil mixture at the concentration 0.01g/100ml water caused 96.67% mortality of 3rd and 4th instar *Ae. aegypti* larvae (Lin 2017). This finding revealed that Lemon essential oil (0.01g/100ml) was found 100% mortality had same potency with the present study of orange peels essential oil and these two were higher potency than the *Ocimum basilicum* leaves essential oil (0.1g/100ml) (Mya et al., 2023). Sharma et al., (2005) reported that the acetone extract of *Nerium indicum* and *Thuja oriertelis* has been studied with LC_{50} values of 200.87, 127.53, 209.00 and 155.97ppm against 3rd instar larvae of *An. stephensi* and *Cx. quinquefasciatus*, respectively. Lyophilized powders of purified CytlA crystal of *B. thuringiensis* were much more

toxic yielding a 50% LC_{50} of 11.332g/L, respectively (Kuppusamy & Ayyadurai 2012). Non polar extract fraction from *Citrus hystrix* is more toxic and is an Effective bio-larvicide with LC_{90} =2885ppm compared with polar extract fraction from *Citrus hystrix* which has an LC_{90} = 3180ppm (Ansari et al., 2000). Maung Maung Mya and his associates revealed that *Citrus hystrix* DC (Kaffir lime) leaves, fruit and fruit peel extracts were found to be very effective and high mortality against 3rd and 4th instar larvae 2.4% and 2.1% concentrated *Citrus hystrix* DC leaves extract in distilled water caused 99.5% and 85.5% mortality and LC_{50} and LC_{90} values were 1.73% and 2.08% concentrations for leaves extract.0.0138g and 0.0515g for fruit extract, 0.0142g and 0.0522g for peel extract [Mya et al., 2015, 2016, 2023). Third and fourth instars *Ae. aegypti* larvae from Pindaya Township were more susceptible, 100% mortality at 0.003125g and 0.00625g in *Citrus curantifolia* and *Citrus maxima* than North Dagon strain (97-100% mortality at 0.00625g) to *Citrus maxima* and *Citrus curantifolia* peel essential oil extracts (Mya et al., 2019). Singhi et al., (2006) have reported that the latex of *Citrus procera* has shown larvicidal efficacy against all three important vector species as *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus* in India. Other researcher evaluated that larvicidal activity of *Plumbago zeylanica* and *Cestrum nocturnum* extracts on *Ae. aegypti*; the LC_{50} values of both medicinal plants were less than 50ppm. The larvicidal stability of the extracts at five constant temperatures (19°C, 22°C,25°C,28°C, and 31°C) evaluated against 4th instars larvae revealed that toxicity of both plant extracts increases in temperature. Halim reported that the insecticidal activity of *Zingiber officinale* against the larval maturation, and adult emergency of *Anopheles pharoensis* 3rd stage showed 100% larval mortality rate and at 0.2% and 0.1% caused mortality of 66.7% respectively (Halim 2008).

In the present study, no cute toxicity effect of *Citrus reticulata* peels essential oil dose 2000mg/kg and 5000mg/kg body weight on albino mice model and no irritation was found essential oil dose 0.04g/ml or 0.000128g/cm^2 on Rabbit skin model for 14 days of trials. Other researchers also revealed that from Sri Lanka and Myanmar they revealed that the *Artemisia vulgaris* extract, Orange, Lemon and *Citrus hystrix* DC, *Citrus maxima* and *Citrus curantifolia* peel essential oil extracts and Neem leaves and seed oil extracts were tolerated well by mice (icr) a period of 14 days (Mya et al., 2016, 2019, 2018, Lin 2017).

Repellency study of *Citrus reticulata peels* essential oil applied to arrive at a cumulative dose for 100% protection was 0.04g/ml or 0.000128g/cm^2 and complete protection time of Orange peels essential oil against *Aedes* mosquito bite was 80 to 100% protection of *Ae. aegypti* mosquito bite for 210 minutes. 100% of protection of mosquito bite was found 120 minutes and over 90% protection was observed 150 minutes. And in semi field trial also found 100% protect from *Aedes* mosquito bite in daytime was 4 hours in indoor. Other researcher mentioned that protection dose and protection time are same with the *Citrus hystrix* DC extract and *Ocimum bosilicum* leaves essential oil, although Orange 0.05g and Lemon 0.05g mixture of essential oil dose 0.08g/ml or 0.0003g/cm^2 gave over 80% protection of bite for 150minutes was lower than the present study (Mya et al., 2016, 2023, Lin 2017). The ethanolic extracts of the Orange peel (*Citrus sinensis*) was tested for the toxicity effect on the larvae of the yellow fever mosquito *Ae. aegypti* (Amusan et al., 2005) susceptibility tests were carried out in *Cx. quinquefasciatus* larvae using peel oil extracts of *Citrus aurantium, Citrus sinensis* and *Citrus limon* (Mwaiko 1992); volatile extracts of *Citrus sinensis* had insecticidal activity against mosquito, cockroach and housefly (Ezeomu et al., 2001). Garcia and Desrochers observed that appreciable mortality only with high concentrations

(1x107cells/ml) of *Bthurin giensis var*. *israelensis* (Garcia & Desrocher 1979). Ansari et al., (2000) reported that the peppermint oil gave 94.1% protection for 6 hours, while mylol oil give 95% protection for 7.2 hours. They also reported that mylol oil and peppermint oil gave 100% protection for 11hours against *An. annularis*. Mylol oil gave 95.4% protection for 8.7 hours for the *Anopheles* species, whereas the peppermint oil gave 86.3% protection for 8 hours. Phukan and Kalita (2005) showed that *Litsea salicifilia* recorded 70% and 50% repellency for 3 and 4 hours, respectively, against *Aedes aegypti*, but they failed to show much activity *against Cx. quinquefasciatus*. Other researcher reported that the akin repellent test at 1.0,2.5 and 5.0 mg/cm^2 concentration gave the mean complete protection time ranged from 119.17 to 387.83 minutes against *An. stephensi* with the benzene, petroleum ether, ethyl acetate and methanol extracts of *Citrullus vulgaris* tasted. Another study of *Citrus reticulate* (Orange peels) and *Citrus limonum* (Lemon) peels extracts have significant repellent activity against *Ae. aegypti* mosquitoes (Lin 2017). Mensah and his party revealed that *Citrus limom* showed that the highest repellent activity of 95%, 92.5% *Citrus aurantifolia* and 82.5% for *Citrus sinensis*, the oils had very strong repellent activities against carpenter ants (Mensah et al., 2014).

5. Conclusion

In conclusion, the present study clearly proved that the efficacy of *Citrus reticulata* orange peel essential oil can be suggested as a larvicidal, ovicidal and repellent activity against *A. aegypti* as target species of mosquitoes. The results reported that the highest dose 0.01g of orange peels essential oil produced 100% knockdown within 60minute and 100% mortality within 24hrs respectively and 100% ovicidal effect as well as persistency was observed. And very low amount of effective lethal concentrations LC_{50} and LC_{90} values were observed. 100% protection of *Aedes* mosquito landing to probe the skin was found 0.04g/ml or 0.000128g/cm^2 of essential oil. Repellency activity of complete protection time was observed over 80% protection for 210minutes and 100% prevention for 120mins and Simi field trial found 100% prevention to bite for 3 hours, Therefore, higher efficacy for controlling mosquitoes and larvicidal, ovicidal and repellent properties of natural product *Citrus reticulata* peel essential oil since they are considered as environmentally safe and eco-friendly approaches for the vector control programmes. In future, study of mosquitocidal properties should be done. *Citrus reticulata* peel essential oil should be tried to control *Ae. aegypti* larvae in water storage containers in natural condition. And also should be formulate the repellency product to use in community to prevent mosquito bite.

Declarations

Source of Funding

This study did not receive any grant from funding agencies in the public, commercial, or not–for–profit sectors.

Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this study.

References

[1] Service, M.W. (1993). Mosquitoes (Culicidae). In Medical Insects and Arachnids, Lane RP, Crosskey R.W. (Eds.), London, Chapman and Hall, 723.

[2] Kyi, K.M. (1970). Malaria vectors in Burma *Anopheles balabacensis balabacensis* Baisas, 1936. Union Bur J. Life Sci., 3: 217–225.

[3] Mya, M.M., Oo, N.S.E., Oo, C.C., Maung, K.S., & Oo, N.N. (2021). Occurrence of avian malaria parasites in bird population in Hpa-an Township Kayin State, Myanmar. Journal of Biological Engineering Research and Review, 8(1): 01–08.

[4] WHO (2023). Diseases Outbreak News; Dengue-Global Situation. Available at http/www.who.int/ emeregencies/diseaseoutbrake-news/item/2023-don518.

[5] Nathan, S.S., Kalaivani, K., & Sehoon, K. (2006). Effects of *Dysoxylum malabaricum* Bedd. (Meliaceae) extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). Biores. Technol., 97: 2077–2083.

[6] Ministry of Health (2020). Communicable diseases, Health in Myanmar. Ministry of Health.

[7] Department of Public Health (2016). Annual report health statistic. DOPH.

[8] Vector Borne Diseases Control (2020). Annual report. VBDC.

[9] Kim, S.I., Shin, O.K., Song, C., Cho, K.Y., & Ahn, Y.J. (2001). Insecticidal activities of aromati22.c plant extracts against four agricultural insects. Agricultural Chemistry and Biotechnology, 44: 23–26.

[10] Warikoo, R., Wahab, N., & Kumar, S. (2011). Oviposition-altering and ovicidal potentials of five essential oils against female adults of the dengue vector, *Aedes aegypti* L. Parasitol Res., 109: 1125–31. doi: 10.1007/s00436-011-2355-y.

[11] Mya, M.M, Chit, M.M., Mitchell, S., Gyi, M.M., & Oo, T. (2011). Community-based control of *Aedes aegypti* larvae by using *Toxorhynchites* larvae in selected townships of Yangon Division, Myanmar. Myanmar Health Sciences Research Journal, 23(2): 101–107.

[12] Mya, M.M., Oo, N.N., Kyi, N.T.T., Kyi, M.M., Thaung, S., & Maung, Y.N.M. (2016). Larvivorous effect of *Aplocheilus panchax* and *Trichogaster trichopterus* native fish of Hpa-an Township, Kayin State. Myanmar Health Sciences Research Journal, 28(2): 121–127.

[13] Mya, M.M., Lwin, N.W., Bo, B., & Gyi, M.M. (2013). Control of *Aedes* larvae in Household Water storage containers using Dragonfly nymphs in Thakayta Township, Yangon Region. Myanmar Health Sciences Research Journal, 25(3): 166–172.

[14] Ogunlade, S.T., Adeshina, I.A., Emma, S.M., & Michael, T.M. (2022). Modelling the ecological dynamics of mosquito populations with multiple co-circulating Wolbachia strains. Scientific Reports, 12: 20826. https://doi.org/10.1038/s41598-022-25242-x.

[15] Huang, M., Tang, M., Yu, J., & Zheng, B. (2020). A stage structured model of delay differential equations for Aedes mosquito population suppression. Discrete Contin. Dyn. Syst., 40: 3467–3484.

[16] Gazali, G.E., Khalid, H.E., Tohami, M., Abdalla, W., & Yagi, S. (1988). Medicinal plants commonly used in Khartoum State. National Centre for Research, Khartoum.

[17] Anthony, Y., & Service, M.W. (1983). Pest and Vector Management in the Tropics. London: Longman.

[18] Ascher, K.R.S., Schmutterer, H., Zebitz, C.P.W., & Naqvi, S.N.H. (1995). The Persian lilac or chinaberry tree: *Melia azedarach* L. In Schmutterer, H. (Eds.), The neem tree: source of unique natural products for integrated pest management, medicine, industry and other purposes, VCH, Weinheim, Pages 605–642.

[19] Nathan, S.S, Chung, P.G., & Murugan, K. (2004). Effect of botanical insecticides and bacterial toxins on the gut enzyme of the rice leaf folder *Cnaphalocrocis medinalis*. Phytoparasitica, 32: 433–443.

[20] Nathan, S.S., & Kalaivani, K. (2005). Efficacy of nucleopolyhedrovirus and azadirachtin on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Biol Control, 34: 93–98.

[21] Nathan, S.S., Kalaivani, K., Murugan, K., & Chung, P.G. (2005b). Efficacy of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae) the rice leaffolder. Crop Prot., 24(8): 760–763.

[22] Nathan, S.S., Kalaivani K., Murugan K., & Chung, P.G. (2005c). The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) the rice leaf folder. Pestic Biochem Physiol., 81: 113–122.

[23] Wandscheer, C.B., Jonny, E.D., Mario, A.N., Fukuyama., da Silva, Yoshiyasu, Jonathan L., Wohlke, J.A., & Fontana José, D. (2004). Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. Toxicon., 8: 829–835.

[24] Brown, A.W.A. (1986). Insecticide resistance in mosquitoes; a pragmatic review. J Am Mosq Control Assoc., 2: 123–140.

[25] Sukumar, K., Perich, M.J., & Boobar, L.R. (1991). Botanical derivatives in mosquito control: A review. J Am Mosq Control Assoc., 7(2): 210–237.

[26] Gimletle, J.D., & Thomson, H.W. (1983). A Dictionary of Malayan Medicine. Oxford University Press, Kuala Lumpur.

[27] Yeung, H.C. (1985). Handbook of Chinese Herbs and Formulas. Institute of Chinese Medicine, Los Angeles.

[28] Kiran, R.S., Bhavani, K., Devi, S.P., Rao, R.B.R., & Reddy, J.K. (2006). Composition and larvicidal activity of leaves and stem essential oils of *Chloroxylon swietenia* D.C. against *Aedes aegypti* and *Anopheles stephensi*. Bioresource Technol., 97: 2481–2484.

[29] Mya, M.M, Aye, Y., Oo, A.W., & Saxena, R.K. (2015). Effect of *Citrus hystrix* DC Leaves Ethanol Extract on larvae of *Aedes aegypti*. Journal of Biological Engineering Research and Review, 2(2): 01–06.

[30] Ibrahim, J., Abu, S.A., Abdul, R.A., Nor, A.M.A., & Norsiha, A. (1996). Chemical composition some Citrus oils from Malaysia. J. Essent. Oil Res., 8: 627–632.

[31] Dan, B., Steven, C., Erich, S., & Andrew, G. (2004). Chinese herbal medicine. Materia Medica, Eastland Press, USA.

[32] Rampa, R., & Prachong, P. (1994). Illustrated keys to the medically important mosquitoes of Thailand. Southeast Asian Journal of Tropical Medicine and Public Health, 25(1): 1–66.

[33] World Health Organization (2005). Guidelines for laboratory and field testing of mosquito larvicides. WHO Geneva 2005.

[34] Finney (1971). Probit Analysis. Third Ed., Cambridge University Press, Cambridge.

[35] Ranjit, S., & Kissoon, N. (2011). Dengue hemorrhagic fever and shock syndromes. Pediatric Critical Car. doi: 10.1097/pcc.0b013e3181e911a7.

[36] Ferreira, D.L., & Camara, L. (2018). Natural vertical transmission of dengue virus in *Aedes aegypti* and *Aedes albopictus*: a systematic review. Parasites & Vectors, 11: 77. doi: 10.1186/s13071-018-2643-9.

[37] Mya, M.M., Oo, A.W., Htay, T.M., New, C.T., Mu, T.Z.N., Aung, Z.Z, Myint, Y.Y., & Thaung, S. (2023). Larvicidal, Ovicidal and Repellency Properties of the Indigenous Plants *Ocimum basilicum* L Leaves Essential Oil against *Aedes aegypti* Mosquitoes. Global Journal of Materials Science and Engineering, 5(1): 133–143. http://doi.org/10.36266/gjmse/133.

[38] Bowers, W.S., Sener, B., Evans, P.H., Bingol, F., & Erdogan, I. (1995). Activity of Turkish medicinal plants against mosquitoes *Aedes aegypti* and *Anopheles gambiae*. Insect Sci Appl., 16(3/4): 339–342.

[39] Govindarajan, M., Jebanesan, A., Pushpanathan, T., & Samidurai, K. (2008a). Studies on effect of *Acalyoha indica* L. (Euphorbiaceae) leaf extract on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitology Research, 103(3): 691–695. https://doi.org/10.1007/s12639-016-0869-z.

[40] Govindarajan, M., Jebanesan, A., & Pushpanathan, T. (2008b). Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. Parasitology Research, 102(2): 289–292. doi: 10.1007/s00436-007-0761-y

[41] Niraimathi, S., Balaji, N., Venkataramanan, N., & Govindarajan, M. (2010). Larvicidal activity of alkaloid from *Sida acuta* against *Anopheles subpictus* and *Culex tritaeniorhynchus*. Int J Curr Res., 11: 034–038.

[42] Shaalan, E.A.S., Deon, C., Mohamed, W.F.Y., Hoda, A.W., & Abdel-Hamid, M. (2005). A review of botanical phytochemicals with mosquitocidal potential. Environment International, 31(8): 1149–1166.

[43] Rajkumar, S., & Jebanesan, A. (2004). Mosquitocidal activities of octasane from *Moschosma polystachyum* Linn. (Lamiaceae). J Ethnopharm., 90: 87–89.

[44] Lin, S.H.T. (2017). Preparation and Characterization of mosquito repellents from Citrus peels. Department of Industrial Chemistry, University of Yangon, Master of Research Thesis.

[45] Sharma, P., Mohan, L., & Srivastava, C.N. (2005). Larvicidal potential of *Nerium indicum* and *Thuja oriertelis* extracts against malaria and Japanese encephalitis vector. J Environ Biol., 26(4): 657–660.

[46] Kuppusamy, C., & Ayyadurai, N. (2012). Synergistic activity of Cyt1A from *Bacillus thuringiensis* subsp. israelensis with *Bacillus sphaericus* B101 H5a5b against *Bacillus sphaericus* B101 H5a5b-resistant strains of *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res., 110: 381–388.

[47] Ansari, M.A., Razdan, R.K., Tandon, M., & Vasudevan, P. (2000). Larvicidal and Repellent actions of *Dalbergia sisoo* Roxb. (F. Leguminosae) oil against mosquitoes. Bioresource Technol., 73: 207.

[48] Mya, M.M., Aung, Z.Z., Lwin, K.K., New, C.T., Oo, A.W., Htay, T.M., Thaung, S., Phyu, K.P., & Maung, Y.N.M. (2019). Larvicidal properties of *Citrus hystrix* DC fruit (Taw-Shaut-Khar-thee) extracts against *Aedes aegypti* larvae collected from different Townships of Yangon. Myanmar Health Sciences Research Journal, 31(1): 23–29.

[49] Singhi, M., Joshi, V., & Dam, P.K. (2006). Studies on *Calotropis procera* as larvicidal and repellent plant against vectors of dengue and DHF in Rajasthan, India. Annual Report 2005–06. Desert Medicine Research Center, Jodhpur, Pages 24–28.

[50] Halim, A.S.A. (2008). Efficacy of *Zingiber officinale* on third stage larvae and adult fecundity of *Musca domestica* and *Anopheles pharoensis*. J Egypt Soc Parasitol., 38: 385–392.

[51] Siti, H.N., Suhaila, M., & Yusof, K. (2022). Potential therapeutic effects of *Citrus hystrix* DC and its Bioactive Compounds on Metabolic Disorders. Pharmaceuticals (Basel), 15(2): 167. doi: 10.3390/ph15020167.

[52] Mya, M.M., Oo, N.N., Oo, A.W., Thaung, S., & Maung, Y.N.M. (2018). Repellency effect of *Artemisia vulgaris* essential oil against *Aedes aegypti* mosquitoes in laboratory condition. Myanmar Health Sciences Research Journal, 30(1): 8–14.

[53] Amusan, A.A.S., Idowu, A.B., & Arowolo, F.S. (2005). Comparative toxicity effect of bush tea leaves (*Hyptis suaveolens*) and orange peel (*Citrus sinensis*) oil extract on larvae of the yellow fever mosquito *Aedes aegypti*. Tanzania Health Research Bulletin, 7: 174–178.

[54] Mwaiko, G.L. (1992). Citrus peel oil extract asmosquito larvae insecticides. East African Medical Journal, 69: 223.

[55] Ezeonu, F.C., Chidume, G.I., & Udedi, S.C. (2001). Insecticidal properties of volatile extracts of orange peels. Bioresource Technology, 76(3): 273–274. https://doi.org/ 10.1016/s0960-8524(00)00120-6.

[56] Garcia, R., & Desrochers, B.D. (1979). Toxicity of B*acillus thuringiensis* var. israelensis to some California mosquitoes under different conditions. Mosq News., 39: 541–544.

[57] Phukan, S., & Kalita, M.C. (2005). Phytopesticidal and repellent efficacy of *Litsea salicifolia* (Lauraceae) against *Aedes aegypti* and *Culex quinquefasciatus*. Indian J Exp Biol., 43: 472–474.

[58] Mensah, F.A., Inkum, E., Agbale, C.M., & Eric, A. (2014). Comparative Evaluation of the Insecticidal and Insect Repellent Properties of the Volatile Oils of *Citrus aurantifolia* (Lime), *Citrus sinensis* (Sweet Orange) and *Citrus limon* (Lemon) on *Camponotus nearcticus* (Carpenter Ants). Int. Journal of Novel Research, 1(2): 19–25.